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     (FILE 'HOME' ENTERED AT 18:53:10 ON 14 AUG 2001)

     FILE 'CAOLD, CAPLUS, CROPU, DGENE, DPCI, ENCOMPPAT, ENCOMPPAT2,
     EUROPATFULL, IFIPAT, INPADOC, JAPIO, PAPERCHEM2, PATDD, PATDPA, PATOSDE,
     PATOSEP, PATOSWO, PCTFULL, PIRA, RAPRA, SYNTHLINE, TULSA, TULSA2,
     USPATFULL, WPIDS' ENTERED AT 18:53:19 ON 14 AUG 2001
L1       870560 S (SACCHARIDE# OR MONOSACCHARIDE# OR OLIGOSACCHARIDE# OR CARBOH
L2       383649 S L1 (L) (PREPAR? OR MAK? OR SYNTH? OR CONV? OR PREP/RL)
L3         5905 S GLYCOSYLTRANSFERASE#
L4          453 S L3 (L) (FUSION PROTEIN# OR CHIMER? PROTEIN# OR (PROTEINS (L)
L5          402 S L2 (L) L4
L6          396 DUP REM L5 (6 DUPLICATES REMOVED)
L7          108 S L6 AND PY<=1997
L8           23 S L7 AND (SUGAR# (W) NUCLEOTIDE#)
L9           10 S L8 AND (REGENER? OR RECYCL?)

=> s l9 and (plant? or microorganism?)
   8 FILES SEARCHED...
  21 FILES SEARCHED...
L10           9 L9 AND (PLANT? OR MICROORGANISM?)
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=> d ibib ab 1-9

L10 ANSWER 1 OF 9 PCTFULL COPYRIGHT 2001 MicroPatent
ACCESSION NUMBER: 1995024495 PCTFULL
TITLE (ENGLISH): TRANSGENIC PRODUCTION OF **OLIGOSACCHARIDES** AND GLYCOCONJUGATES
TITLE (FRENCH): PRODUCTION TRANSGENIQUE D'**OLIGOSACCHARIDES** ET DE GLYCOCONJUGUES
INVENTOR(S): PRIETO, Pedro, Antonio; SMITH, David, Fletcher; CUMMINGS, Richard, Dale; KOPCHIK, John, Joseph; MUKERJI, Pradip; MOREMEN, Kelley, Wilson; PIERCE, James, Michael
PATENT ASSIGNEE(S): ABBOTT LABORATORIES
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9524495	**A1**	**19950914**

DESIGNATED STATES: AU CA FI JP MX NL NZ AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
APPLICATION INFO.: WO 1995-US967 19950124
PRIORITY (ORIGINAL): US 1994-208889 19940309

ABEN The invention relates to the milk of a transgenic non-human mammal. The milk is characterized in that it contains heterologous components produced as the secondary gene products of a heterologous gene contained in the genome of the transgenic non-human mammal. The heterologous gene encodes a heterologous catalytic entity such as a human enzyme selected from the group consisting of **glycosyltransferases**, phosphorylases, hydroxylases, peptidases and sulfotransferases. Especially useful in the practice of the invention are human **glycosyltransferases**. The desired heterologous components include **oligosaccharides**, glycoconjugates. The **oligosaccharides** and glycoconjugates may be isolated from the milk of the transgenic mammals and used in the **preparation** of pharmaceuticals, diagnostic kits, nutritional products and the like. The whole transgenic milk may also be used to formulate nutritional products that provide special advantages. The transgenic milk may also be used in the production of specialized enteral nutritional products.

ABF L'invention se rapporte au lait d'un mammifere transgenique autre que l'homme. Ce lait se caracterise en ce qu'il contient des composants heterologues obtenus sous forme de produits geniques secondaires d'un gene heterologue contenu dans le genome du mammifere transgenique. Le gene heterologue code une entite catalytique heterologue, telle qu'une enzyme humaine selectionnee dans le groupe compose de **glycosyltransferases**, phosphorylases, hydroxylases, peptidases, et sulfotransferases. Des **glycosyltransferases** humaines sont notamment utiles dans la mise en application de l'invention. Les composants heterologues desires comprennent des **oligosaccharides** et des glycoconjugues. Les **oligosaccharides** et les glycoconjugues peuvent etre isoles du lait des mammiferes transgeniques et utilises dans la **preparation** de produits pharmaceutiques, de kits diagnostiques, de produits nutritionnels et analogues. Le lait entier transgenique peut etre egalement utilise pour formuler des produits nutritionnels qui presentent des avantages specifiques. Ce lait transgenique peut aussi etre utilise dans la production de produits nutritionnels enteriques

specialises.

L10 ANSWER 2 OF 9 PCTFULL COPYRIGHT 2001 MicroPatent
ACCESSION NUMBER: 1995024494 PCTFULL
TITLE (ENGLISH): HUMANIZED MILK
TITLE (FRENCH): LAIT HUMANISE
INVENTOR(S): PRIETO, Pedro, Antonio; SMITH, David, Fletcher; CUMMINGS, Richard, Dale; KOPCHIK, John, Joseph; MUKERJI, Pradip; MOREMAN, Kelley, Wilson; PIERCE, James, Michael
PATENT ASSIGNEE(S): ABBOTT LABORATORIES
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9524494	**A1**	**19950914**

DESIGNATED STATES: AU CA FI JP MX NO NZ AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
APPLICATION INFO.: WO 1995-US926 19950124
PRIORITY (ORIGINAL): US 1994-209122 19940309

ABEN The invention relates to humanized milk. The milk is produced by a non-human transgenic mammal wherein the genome of said transgenic non-human mammal contains at least one heterologous gene encoding for a human catalytic entity and wherein the catalytic entity produces **oligosaccharides** and glycoconjugates that are present in the milk of said transgenic non-human mammal. An especially useful catalytic entity is human **glycosyltransferases** which produce **oligosaccharides** and glycoconjugates. A method of obtaining humanized milk is disclosed. The method comprises the steps of (a) inserting into the genome of a non-human mammal a heterologous gene encoding the production of a human catalytic entity wherein said catalytic entity produces a secondary gene product in the milk of said non-human mammal; and (b) milking said non-human mammal. The humanized milk may be used in the **preparation** of an enteral nutritional product useful in the nutritive maintenance of an animal.

ABF L'invention concerne du lait humanise. Le lait est produit par un mammifere transgenique non humain, le genome de ce dernier contenant au moins un gene heterologue codant pour une entite catalytique humaine, laquelle produit les **oligosaccharides** et les glycoconjugues presents dans le lait dudit mammifere. L'entite catalytique particulierement utile est constituee de **glycosyltransferases** humaines produisant des **oligosaccharides** et des glycoconjugues. L'invention concerne egalement un procede de production de lait humanise qui consiste a: (a) introduire dans le genome d'un mammifere non humain un gene heterologue codant la production d'une entite catalytique humaine, laquelle engendre un produit genique secondaire dans le lait dudit mammifere non humain; et (b) a traire ledit mammifere non humain. Ce lait humanise peut etre utilise dans la **preparation** d'un produit nutritif enteral utile dans l'alimentation d'un animal.

L10 ANSWER 3 OF 9 PCTFULL COPYRIGHT 2001 MicroPatent
ACCESSION NUMBER: 1995024488 PCTFULL
TITLE (ENGLISH): TRANSGENIC ANIMALS PRODUCING **OLIGOSACCHARIDES** AND GLYCOCONJUGATES
TITLE (FRENCH): ANIMAUX TRANSGENIQUES PRODUISANT DES **OLIGOSACCHARIDES** ET DES

GLYCOCONJUGUES

INVENTOR(S): PRIETO, Pedro, Antonio; SMITH, David, Fletcher; CUMMINGS, Richard, Dale; KOPCHIK, John, Joseph; MUKERJI, Pradip; MOREMEN, Kelley, Wilson; PIERCE, James, Michael

PATENT ASSIGNEE(S): ABBOTT LABORATORIES

LANGUAGE OF PUBL.: English

DOCUMENT TYPE: Patent

PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9524488	**A1**	**19950914**

DESIGNATED STATES: AU CA FI JP MX NO NZ AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE

APPLICATION INFO.: WO 1995-US1147 19950124

PRIORITY (ORIGINAL): US 1994-209132 19940309

ABEN The invention relates to transgenic non-human mammals characterized in that the genome of said mammals contain at least one heterologous gene encoding for the production of heterologus catalytic entity selected from the group consisting of enzymes and antibodies, and wherein said catalytic entity produces a second heterologous product in the milk of said mammal. Especially useful in the practise of the invention are human **glycosyltransferases** and transgenic sheep, goats and cows. The heterologous product includes **oligosaccharides** and glycoconjugates.

ABF L'invention se rapporte a des mammiferes transgeniques autres que l'homme qui se caracterisent en ce que leur genome contient au moins un gene heterologue codant pour la production d'une entite catalytique heterologue selectionnee dans le groupe compose d'enzymes et d'anticorps, cette entite catalytique formant un second produit heterologue dans le lait du mammifere. Les **glycosyltransferases** humaines ainsi que les moutons, les chevres et les vaches transgeniques sont particulierement utiles dans la mise en application de l'invention. Le produit heterologue comprend des **oligosaccharides** et des glycoconjugues.

L10 ANSWER 4 OF 9 PCTFULL COPYRIGHT 2001 MicroPatent

ACCESSION NUMBER: 1993008205 PCTFULL

TITLE (ENGLISH): PRODUCTION OF FUCOSYLATED **CARBOHYDRATES** BY ENZYMATIC FUCOSYLATION **SYNTHESIS** OF **SUGAR NUCLEOTIDES**; AND IN SITU **REGENERATION** OF GDP-FUCOSE

TITLE (FRENCH): **PREPARATION** D'HYDRATES DE CARBONE FUCOSYLES PAR **SYNTHESE** A FLUCOSYLATION ENZYMATIQUE DE NUCLEOTIDES DE SUCRES, ET **REGENERATION** IN SITU DE GDP-FUCOSE

INVENTOR(S): WONG, Chi-Huey; ICHIKAWA, Yoshitaka; SHEN, Gwo-Jenn; LIU, Kun-Chin

PATENT ASSIGNEE(S): THE SCRIPPS RESEARCH INSTITUTE

LANGUAGE OF PUBL.: English

DOCUMENT TYPE: Patent

PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9308205	**A1**	**19930429**

DESIGNATED STATES: AU BB BG BR CA CS FI HU JP KP KR LK MG MN MW NO PL RO RU SD AT BE CH DE FR GB GR IE IT LU MC NL SE BF BJ CF CG CI CM GA GN ML MR SN TD TG

APPLICATION INFO.: WO 1992-US8789 19921015

PRIORITY (ORIGINAL): US 1991-777662 19911015

	US 1992-901260	19920619
	US 1992-910612	19920708
	US 1992-961076	19921014

ABEN This invention contemplates improved methods of enzymatic production of **carbohydrates** especially fucosylated **carbohydrates**. Improved **syntheses** of glycosyl 1- or 2-phosphates using both chemical and enzymatic means are also contemplated. The phosphorylated glycosides are then used to produce **sugar nucleotides** that are in turn used as donor **sugars** for glycosylation of acceptor **carbohydrates**. Especially preferred herein in the use of a disclosed method for fucosylation.

ABF L'invention se rapporte a des procedes de production enzymatique d'hydrates de carbone, en particulier des hydrates de carbone fucosyles. Des **syntheses** ameliorees de 1- ou 2-phosphates de glycosyle, effectuees a la fois par l'intermediaire de moyens enzymatiques et de moyens chimiques sont egalement decrits. Les glucosides phosphoryles sont ensuite utilises pour produire des nucleotides de sucres qui sont a leur tour utilises comme sucres donneurs pour la glycosylation d'hydrates de carbone accepteurs. L'utilisation d'un procede de fucosylation decrit est particulierement preferee.

L10 ANSWER 5 OF 9 PCTFULL COPYRIGHT 2001 MicroPatent

ACCESSION NUMBER: 1992016640 PCTFULL

TITLE (ENGLISH): **OLIGOSACCHARIDE** ENZYME SUBSTRATES AND INHIBITORS: METHODS AND COMPOSITIONS

TITLE (FRENCH): **OLIGOSACCHARIDES** SERVANT DE SUBSTRATS ET D'INHIBITEURS D'ENZYMES: PROCEDES ET COMPOSITIONS

INVENTOR(S): WONG, Chi-Huey; ICHIKAWA, Yoshitaka; SHEN, Gwo-Jenn

PATENT ASSIGNEE(S): THE SCRIPPS RESEARCH INSTITUTE

LANGUAGE OF PUBL.: English

DOCUMENT TYPE: Patent

PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9216640	**A1**	**19921001**

DESIGNATED STATES: AT AU BE CA CH DE DK ES FR GB GR IT JP LU MC NL SE

APPLICATION INFO.:	WO 1992-US2178	19920317
PRIORITY (ORIGINAL):	US 1991-670701	19910318
	US 1991-707600	19910520
	US 1991-738211	19910730
	US 1992-852409	19920316

ABEN **Oligosaccharide** compounds that are substrates and inhibitors of **glycosyltransferase** and glycosidase enzymes and compositions containing such compounds are disclosed. A method of glycosylation is also disclosed. An E. coli transformed with phagemid CMPSIL-1, which phagemid comprises a gene for a modified CMP-sialic acid **synthetase** enzyme, which transformed E. coli has the ATCC accession No. 68531 is also provided.

ABF Composes **oligosaccharides** constituant des substrats et des inhibiteurs des enzymes **glycosyltransferase** et glycosidase et composition contenant ces composes. On decrit egalement un procede de glycosylation, ainsi qu'un E. coli transforme avec du phagemide CMPSIL-1, lequel phagemide comprend un gene pour l'enzyme **synthetase** d'acide CMP-sialique modifiee, l'E. coli transforme ayant recu le numero d'enregistrement ATCC 68531.

L10 ANSWER 6 OF 9 PCTFULL COPYRIGHT 2001 MicroPatent
ACCESSION NUMBER: 1991013988 PCTFULL
TITLE (ENGLISH): RECOMBINANT beta-1,4 GLUCAN **SYNTHASE** PROTEINS
TITLE (FRENCH): PROTEINES RECOMBINANTES DE beta-1,4 GLUCANE **SYNTHASE**
INVENTOR(S): SAXENA, Inder, M., Jr.; LIN, Fong, Chyr; BROWN, R., Malcolm, Jr.
PATENT ASSIGNEE(S): THE BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM; SAXENA, Inder, M., Jr.; LIN, Fong, Chyr; BROWN, R., Malcolm, Jr.
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9113988	**A1**	**19910919**

DESIGNATED STATES: AT AT AU BB BE BF BG BJ BR CA CF CG CH CH CM DE DE DK DK ES ES FI FR GA GR HU IT JP KP KR LK LU LU MC MG ML MR MW NL NL NO PL RO SD SE SE SN SU US
APPLICATION INFO.: WO 1991-US1726 19910314
PRIORITY (ORIGINAL): US 1990-494093 19900315

ABEN The present invention relates to the compositions and methods associated with the cloning of the subunits of beta-1,4 glucan **synthases** responsible for catalyzing beta-1,4 glucan polymer biosynthesis. The invention relates further to compositions and methods for obtaining host cells containing recombinant beta-1,4 glucan **synthases** as well as compositions and methods for obtaining beta-1,4 glucan **synthase** from natural sources. In certain aspects, the present invention provides methods for the cloning of both the 83 Kd subunit and the 93 Kd subunit of the cellulose **synthase** enzyme from Acetobacter xylinum.

ABF Compositions et procedes associes au clonage de sous-unites de beta-1,4 glucane **synthase** servant a catalyser la biosynthese du polymere beta-1,4 glucane. La presente invention a egalement trait a d'autres compositions et procedes permettant d'obtenir des cellules hotes contenant des beta-1,4 glucane **synthases** recombinantes et a des compositions et procedes permettant d'obtenir la beta-1,4 glucane **synthase** de sources naturelles. Sous certains aspects, la presente invention presente des procedes pour le clonage tant de la sous-unite 83 Kd et de la sous-unite 93 Kd de l'enzyme cellulose **synthase** a partir de Acetobacter xylinum.

L10 ANSWER 7 OF 9 USPATFULL
ACCESSION NUMBER: 97:120488 USPATFULL
TITLE: Methods of making transgenic animals producing oligosaccharides and glycoproteins
INVENTOR(S): Prieto, Pedro Antonio, Columbus, OH, United States
Smith, David Fletcher, Athens, GA, United States
Cummings, Richard Dale, Edmond, OK, United States
Kopchick, John Joseph, Athens, OH, United States
Mukerji, Pradip, Gahanna, OH, United States
Moremen, Kelley Wilson, Athens, GA, United States
Pierce, James Michael, Athens, GA, United States
PATENT ASSIGNEE(S): Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

NUMBER	KIND	DATE

PATENT INFORMATION: US 5700671 19971223 <--
APPLICATION INFO.: US 1995-434151 19950502 (8)
RELATED APPLN. INFO.: Division of Ser. No. US 1994-209132, filed on 9 Mar 1994
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Chambers, Jasemine C.
ASSISTANT EXAMINER: Crouch, Deborah
LEGAL REPRESENTATIVE: Becker, Cheryl L.
NUMBER OF CLAIMS: 8
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 19 Drawing Figure(s); 15 Drawing Page(s)
LINE COUNT: 1805
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to transgenic non-human mammals characterized in that the genome of said mammals contain at least one heterologous gene encoding for the production of heterologous catalytic entity selected from the group consisting of enzymes and antibodies, and wherein said catalytic entity produces a second heterologous product in the milk of said mammal. Especially useful in the practice of the invention are human glycosyltransferases and transgenic sheep, goats and cows. The heterologous product includes oligosaccharides and glycoconjugates.

L10 ANSWER 8 OF 9 USPATFULL
ACCESSION NUMBER: 97:3736 USPATFULL
TITLE: Oligosaccharide enzyme substrates and inhibitors: methods and compositions
INVENTOR(S): Wong, Chi-Huey, San Diego, CA, United States
Ichikawa, Yoshitaka, San Diego, CA, United States
Shen, Gwo-Jenn, Carlsbad, CA, United States
PATENT ASSIGNEE(S): The Scripps Research Institute, La Jolla, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5593887		19970114	<--
APPLICATION INFO.:	US 1995-476685		19950607 (8)	

RELATED APPLN. INFO.: Division of Ser. No. US 1994-219242, filed on 29 Mar 1994, now patented, Pat. No. US 5461143 which is a continuation-in-part of Ser. No. US 1992-852409, filed on 16 Mar 1992, now abandoned which is a continuation-in-part of Ser. No. US 1991-738211, filed on 30 Jul 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-670701, filed on 18 Mar 1991, now patented, Pat. No. US 5278299 And Ser. No. US 1991-707600, filed on 30 May 1991, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Fleisher, Mindy
ASSISTANT EXAMINER: Weiss, Bonnie D.
LEGAL REPRESENTATIVE: Welsh & Katz, Ltd.
NUMBER OF CLAIMS: 4
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 3 Drawing Figure(s); 3 Drawing Page(s)
LINE COUNT: 3572
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Oligosacaharide compounds that are substrates and inhibitors of glycosyltransferase and glycosidase enzymes and compositions containing such compounds are disclosed. A method of glycosylation is also disclosed. An E. coli transformed with phagemid CMPSIL-1, which phagemid comprises a gene for a modified CMP-sialic acid synthetase enzyme, which transformed E. coli has the ATCC accession No. 68531 is also provided.

L10 ANSWER 9 OF 9 USPATFULL

ACCESSION NUMBER: 95:95009 USPATFULL

TITLE: Oligosaccharide enzyme substrates and inhibitors: methods and compositions

INVENTOR(S): Wong, Chi-Huey, San Diego, CA, United States
Ichikawa, Yoshitaka, San Diego, CA, United States
Shen, Gwo-Jenn, Carlsbad, CA, United States

PATENT ASSIGNEE(S): The Scripps Research Institute, La Jolla, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5461143		19951024	<--
APPLICATION INFO.:	US 1994-219242		19940329	(8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1992-889652, filed on 26 May 1992, now abandoned which is a continuation-in-part of Ser. No. US 1992-852409, filed on 16 Mar 1992, now abandoned which is a continuation-in-part of Ser. No. US 1991-738211, filed on 30 Jul 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-670701, filed on 18 Mar 1991, now patented, Pat. No. US 5278299 And a continuation-in-part of Ser. No. US 1991-707600, filed on 30 May 1991, now abandoned

DOCUMENT TYPE: Utility

FILE SEGMENT: Granted

PRIMARY EXAMINER: Robinson, Douglas W.

ASSISTANT EXAMINER: Fonda, Kathleen Kahler

LEGAL REPRESENTATIVE: Welsh & Katz, Ltd.

NUMBER OF CLAIMS: 11

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 3 Drawing Figure(s); 3 Drawing Page(s)

LINE COUNT: 3735

AB Oligosaccharide compounds that are substrates and inhibitors of glycosyltransferase and glycosidase enzymes and compositions containing such compounds are disclosed. A method of glycosylation is also disclosed. An E. coli transformed with phagemid CMPSIL-1, which phagemid comprises a gene for a modified CMP-sialic acid synthetase enzyme, which transformed E. coli has the ATCC accession No. 68531 is also provided.

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                E E3+ALL
L2          30992 SEA ABB=ON  PLU=ON  FUSION PROTEIN# OR CHIMER? PROTEIN# OR
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                E E3+ALL
L3           3206 SEA ABB=ON  PLU=ON  GLYCOSYLTRANSFERASE#
L4             37 SEA ABB=ON  PLU=ON  L2 (L) L3
L5             20 SEA ABB=ON  PLU=ON  L4 AND PD<19971118
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L6         388115 SEA ABB=ON  PLU=ON  (SACCHARIDE# OR MONOSACCHARIDE# OR
                OLIGOSACCHARIDE# OR CARBOHYDRATE# OR POLYSACCHARIDE# OR
                SUGAR#)
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L8              1 SEA ABB=ON  PLU=ON  L7 (L) L2 (L) L3
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=> d ibib ab 1-20

L5 ANSWER 1 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:706981 HCAPLUS
DOCUMENT NUMBER: 128:46181
TITLE: .beta.1,4 N-acetylgalactosaminyltransferase (GM2/GD2/GA2 synthase) forms homodimers in the endoplasmic reticulum: a strategy to test for dimerization of Golgi membrane proteins
AUTHOR(S): Zhu, Guofen; Jaskiewicz, Ewa; Bassi, Rosaria; Darling, Douglas S.; Young, William W., Jr.
CORPORATE SOURCE: Departments of Biological and Biophysical Sciences and Biochemistry, Schools of Dentistry and Medicine, University of Louisville, Louisville, KY, 40292, USA
SOURCE: Glycobiology (**1997**), 7(7), 987-996
CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Many Golgi membrane-bound **glycosyltransferases** exist as intermol. disulfide bonded species, some of which have been demonstrated to be homodimers. Evidence for homodimer formation has come primarily from radiation inactivation expts. We utilized an alternative strategy to test for homodimer formation of the cloned .beta.1,4 N-acetylgalactosaminyltransferase (GalNAcT) responsible for synthesis of the glycosphingolipids GM2, GD2, and GA2. We stably transfected CHO cells with myc epitope-tagged GalNAcT, which localizes primarily to the Golgi, and a hemagglutinin (HA) epitope-tagged GalNAcT **fusion protein** in which the cytoplasmic domain of GalNAcT was replaced by an ER retention signal. We then sought evidence for dimer formation between the two forms of GalNAcT. Immunopptn. with anti-myc or anti-HA co-immunopptd. the HA-tagged form or the myc-tagged form, resp., providing evidence for the phys. assocn. of the two forms of GalNAcT. As a result of this assocn., GalNAcT/myc increased in the ER as demonstrated by Western blots and immunofluorescence. The rapid formation of dimers provided further evidence for dimer formation occurring in the ER. In summary, these results demonstrate that GalNAcT forms homodimers as a result of intermol. disulfide bond formation in the ER. Furthermore, this ER motif strategy is potentially useful for demonstrating homodimer formation of other Golgi enzymes.

L5 ANSWER 2 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:448100 HCAPLUS
DOCUMENT NUMBER: 127:61642
TITLE: expression of genes for mammalian glycosyltransferases for alteration of patterns of protein glycosidation in fungal hosts
PATENT ASSIGNEE(S): Ernst, Joachim, Germany
SOURCE: Ger. Offen., 7 pp.
CODEN: GWXXBX
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 19546680	A1	19970619	DE 1995-19546680	19951214 <--

AB A method of altering the patterns of glycosidation of **proteins** manufd. in a yeast host by expression of genes for mammalian **glycosyltransferases** is described. The mammalian genes may included in **chimeric** genes with those for fungal **glycosyltransferase**, e.g to ensure targetting of the enzyme to the correct organelle. A **chimeric** gene for a **fusion**

protein of a human galactosyltransferase and a yeast mannosyltransferase using the anchor domain of the mannosyltransferase was constructed by std. methods and expressed in Saccharomyces cerevisiae using the GAL10 promoter. The galactosyltransferase manufd. in yeast had the same Km (44 .mu.M) as the human form for UDP-galactose and had a Vmax of 0.184 vs. 0.7 .mu.mol/min/mg protein.

L5 ANSWER 3 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:312571 HCAPLUS
DOCUMENT NUMBER: 126:316364
TITLE: Production of antibody to detect and purify cyclodextrin glycosyltransferase and over-production of its soluble proteins using gene fusion and controlled environment (starch, CGT)
AUTHOR(S): Han, Nam Soo
CORPORATE SOURCE: Purdue Univ., West Lafayette, IN, USA
SOURCE: (**1996**) 171 pp. Avail.: Univ. Microfilms Int., Order No. DA9713519
From: Diss. Abstr. Int., B 1997, 57(11), 6676
DOCUMENT TYPE: Dissertation
LANGUAGE: English
AB Unavailable

L5 ANSWER 4 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:281159 HCAPLUS
DOCUMENT NUMBER: 126:260139
TITLE: Human **glycosyltransferase** or fucosyltransferase **fusion proteins**, **chimeric** genes encoding them, and expression in host cell
INVENTOR(S): Lowe, John B.; Legault, Daniel J.
PATENT ASSIGNEE(S): Regents of the University of Michigan, USA
SOURCE: PCT Int. Appl., 329 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
WO 9709421	A1	19970313	WO 1996-US13816	19960906	<--
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM					
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM					
US 5770420	A	19980623	US 1995-525058	19950908	
AU 9669035	A1	19970327	AU 1996-69035	19960906	<--
PRIORITY APPLN. INFO.:			US 1995-525058	19950908	
			WO 1996-US13816	19960906	

AB A method for isolating a gene, comprising: (1) isolating a cell possessing a post-translational characteristic of interest, said post-translational characteristic being the presence of a membrane-bound oligosaccharide or polysaccharide of interest on the surface of said cell, the presence of a sol. oligosaccharide or polysaccharide of interest in an ext. of said cell, or the presence of a particularly **glycosyltransferase** activity in an ext. of said cell; (2) creating a genetic library of either cDNA or genomic DNA from the genetic material of said isolated cell; (3) transforming host cells with said genetic library; and (4) screening said transformed host cells for a host cell contg. said post-translational characteristic, thereby obtaining a cell contg. said gene, is disclosed.

The method can be used to obtain genes encoding **glycosyltransferases**. Esp. plasmid vectors for expression of fucosyltransferase **fusion proteins** are described. Many alternative fucosyltransferase III-fucosyltransferase TVI **chimeric** genes are included.

L5 ANSWER 5 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:175169 HCAPLUS
DOCUMENT NUMBER: 126:168883
TITLE: An in vivo approach for the identification of acceptor sites for O-glycosyltransferases: Motifs for the addition of O-GlcNAc in Dictyostelium discoideum
AUTHOR(S): Jung, Eva; Gooley, Andrew A.; Packer, Nicolle H.; Slade, Martin B.; Williams, Keith L.; Dittrich, Werner
CORPORATE SOURCE: MUCAB (Macquarie University Center for Analytical Biotechnology) School of Biological Sciences, Macquarie University, Sydney, 2109, Australia
SOURCE: Biochemistry (**1997**), 36(13), 4034-4040
CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB To identify and analyze acceptor sequences for O-glycosylation, we have developed an in vivo system expressing short peptides as glutathione S-transferase fusion proteins in the eukaryotic host Dictyostelium discoideum. Using this approach, we show that a short peptide motif (PTVTPT), present in the D. discoideum cell-surface glycoprotein PsA, is sufficient as a signal for O-glycosylation, even when fused to a heterologous protein. Monosaccharide anal. and solid-phase protein sequencing showed that the modification is a single N-acetylglucosamine attached to threonine residues. This was further confirmed by electrospray-mass spectrometry. The O-linked glycosylation of both this peptide and authentic PsA presents the modB-dependent carbohydrate-specific epitope identified by the monoclonal antibody MUD50. Substitution of threonine by serine residues in this peptide also yields a glycosylated fusion protein which is modified with single N-acetylglucosamine residues, but not all of the serines are glycosylated.

L5 ANSWER 6 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:137084 HCAPLUS
DOCUMENT NUMBER: 126:234768
TITLE: Golgi localization of glycosyltransferases: more questions than answers
AUTHOR(S): Colley, Karen J.
CORPORATE SOURCE: College of Medicine, University of Illinois, Chicago, IL, 60612, USA
SOURCE: Glycobiology (**1997**), 7(1), 1-13
CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with .apprx.55 refs. The structures of cellular oligosaccharides are detd. by a series of processing reactions catalyzed by Golgi glycosidases and **glycosyltransferases**. While there are subtle cell type differences in Golgi enzyme subcompartmentation, in general, glycosylation enzymes are localized within the Golgi cisternae in the same sequence in which they act to modify oligosaccharide substrates. The possibility that this enzyme subcompartmentation may control the types of oligosaccharides expressed by a cell has led to an interest in the signals and mechanisms directing enzyme localization in the Golgi cisternae. All glycosidases and **glycosyltransferases** characterized thus far have very little sequence homol. that might suggest a common Golgi retention signal, but they do share a similar domain structure. They are all type II transmembrane **proteins** consisting of an amino

terminal cytoplasmic tail, a signal anchor transmembrane domain, a stem region, and a large luminal catalytic domain. Their lack of sequence homol. suggests that these **proteins'** Golgi retention signals are not linear amino acid sequences, but most likely involve general characteristics or conformations or larger protein domains. The peptide sequences required for Golgi retention of the N-acetylglucosaminyltransferase I (GlcNAcTI), .beta.1,4-galactosyltransferase (GalT) and .alpha.2,6-sialyltransferase (ST) have been extensively studied. To do this, researchers created mutant and **chimeric proteins**, expressed these in tissue culture cells, and localized these **proteins** using immunofluorescence microscopy. The cell surface expression of deletion mutants suggested that the deleted sequences were necessary for Golgi retention. Then, if these sequences were fused to an non-Golgi reporter protein and this **chimeric** or hybrid protein was retained in the Golgi, then these sequences were also sufficient for Golgi retention. Due to differences in reporter **proteins** used to construct these **chimeric proteins**, different cell types used for protein expression, different levels of protein expression, and different methods of cell surface protein detection, these expts. have led to somewhat confusing results. However, in general, it appears that the GalT relies primarily on its transmembrane domain for Golgi retention, while the GlcNAcTI and ST have requirements for their transmembrane regions, sequences flanking these regions, and luminal stem sequences. Based on these results, two potential Golgi retention mechanisms have been proposed and are now being tested. The observation that **glycosyltransferase** transmembrane domains are frequently sufficient for Golgi retention has led to the first of these models, the bilayer thickness model. This model proposes that the shorter transmembrane domains of Golgi **proteins** prevent them from entering cholesterol-rich transport vesicles destined for the plasma membrane, and that this leads to Golgi retention. The second of these models is supported by the role of multiple protein domains in the Golgi retention of some **proteins**. This model, the oligomerization/kinetics recognition model of Golgi retention, proposes that the formation of insol. protein homo-oligomers or very large hetero-oligomers prevents protein movement into transport vesicles destined for later compartments. Initial work suggests that the bilayer thickness mechanism may play a role in the retention of some Golgi retained **proteins**; however, it is not the sole retention mechanism. Other evidence suggests that an oligomerization/kinetics recognition mechanism may be more common, but definitive proof for its general use in Golgi protein retention is lacking. More research is required to further elucidate the sequences and particularly the mechanisms of Golgi retention. In the future, the authors hope to be able to explain the cell type differences in glycosylation enzyme Golgi subcompartmentation, the different sequence requirements for the Golgi retention of the same enzyme in various cell types, and whether differences in glycosylation enzyme Golgi subcompartmentation change the types of oligosaccharides made by a cell.

L5 ANSWER 7 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:53962 HCAPLUS
DOCUMENT NUMBER: 126:72038
TITLE: Expression of glycosyltransferase in Aspergillus with increased secretion
INVENTOR(S): Kadoma, Katherine H.; Ward, Michael
PATENT ASSIGNEE(S): Genencor International, Inc., USA
SOURCE: PCT Int. Appl., 61 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
WO 9636718	A1	19961121	WO 1996-US7171	19960516	<--
W: CA, FI, JP, MX					
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE					
CA 2221271	AA	19961121	CA 1996-2221271	19960516	<--
EP 826054	A1	19980304	EP 1996-920281	19960516	
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI					

PRIORITY APPLN. INFO.: US 1995-443954 19950518
WO 1996-US7171 19960516

AB A method for the expression of **glycosyltransferase** in Aspergillus with increased level of secretion is described. The method consists of expression of a chimeric DNA comprised from 5'-terminus of (1) a signal sequence; (2) a DNA sequence encoding a secreted protein of a filamentous fungi, preferably Aspergillus; (3) a DNA sequence encoding a cleavable linker; and (4) a DNA sequence encoding a **glycosyltransferase**. Prepn. of a **fusion protein** consisting of a truncated form of .alpha.-2,3-sialyltransferase and the entire glucoamylase and expression of the **fusion protein** in Aspergillus niger var. awamori were demonstrated.

L5 ANSWER 8 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:40940 HCAPLUS
DOCUMENT NUMBER: 126:85444
TITLE: Cloning of the gene for monogalactosyldiacylglycerol synthase and its evolutionary origin
AUTHOR(S): Shimojima, Mie; Ohta, Hiroyuki; Iwamatsu, Akihiro; Masuda, Tatsuru; Shioi, Yuzo; Takamiya, Ken-ichiro
CORPORATE SOURCE: Fac. Biosci. Biotechnol., Tokyo Inst. Technol., Yokohama, 226, Japan
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (**1997**), 94(1), 333-337
CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Monogalactosyldiacylglycerol (MGDG) synthase (UDPgalactose:1,2-diacylglycerol 3-.beta.-D-galactosyltransferase; EC 2.4.1.46) catalyzes formation of MGDG, a major structural lipid of chloroplast. We cloned a cDNA for the synthase from cucumber cDNA library. The full-length cDNA clone was 2142 bp, and it contains a 1575-bp open reading frame encoding 525 aa. The open reading frame consists of the regions for a mature protein (422 aa; Mr of 46,552) and transit peptide to chloroplast (103 aa). Although the mol. wt. of mature protein region matched that purified from cucumber cotyledons, it was quite different from those purified from spinach (.+-.20 kDa) reported by other groups. The mature region of the protein was expressed in Escherichia coli as a **fusion protein** with glutathione S-transferase. The expression in E. coli showed that the protein catalyzed MGDG synthesis very efficiently. Therefore, we concluded that the cDNA encodes MGDG synthase in cucumber. In addn., the deduced amino acid sequence of the MGDG synthase cDNA showed homol. with MurG of Bacillus subtilis and E. coli, which encode a **glycosyltransferase** catalyzing the last step of peptidoglycan synthesis in bacteria. This sequence homol. implies that the machinery of chloroplast membrane biosynthesis is evolutionarily derived from that of cell wall biosynthesis in bacteria. This is consistent with the endosymbiotic hypothesis of chloroplast formation.

L5 ANSWER 9 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:7801 HCAPLUS
DOCUMENT NUMBER: 126:128654
TITLE: Switching amino-terminal cytoplasmic domains of .alpha.(1,2)fucosyltransferase and .alpha.(1,3)galactosyltransferase alters the

expression of H substance and Gal.alpha.(1,3)Gal

AUTHOR(S): Osman, Narin; McKenzie, Ian F. C.; Mouhtouris, Effie; Sandrin, Mauro S.

CORPORATE SOURCE: Austin Res. Inst., Austin Hosp., Heidelberg, 3084, Australia

SOURCE: J. Biol. Chem. (**1996**), 271(51), 33105-33109
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB When .alpha.(1,2)fucosyltransferase cDNA is expressed in cells that normally express large amts. of the terminal carbohydrate Gal.alpha.(1,3)Gal, and therefore the .alpha.(1,3)galactosyltransferase (GT), the Gal.alpha.(1,3)Gal almost disappears, indicating that the presence of the .alpha.(1,2)fucosyltransferase (HT) gene/enzyme alters the synthesis of Gal.alpha.(1,3)Gal. A possible mechanism to account for these findings is enzyme location within the Golgi app. We examd. the effect of Golgi localization by exchanging the cytoplasmic tails of HT and GT; if Golgi targeting signals are contained within the cytoplasmic tail sequences of these enzymes then a tail switch would permit GT first access to the substrate and thereby reverse the obsd. dominance of HT. Two **chimeric glycosyltransferase proteins** were constructed and compared with the normal **glycosyltransferases** after transfection into COS cells. The **chimeric** enzymes showed Km values and cell surface carbohydrate expression comparable with normal **glycosyltransferases**. Coexpression of the two **chimeric glycosyltransferases** resulted in cell surface expression of Gal.alpha.(1,3)Gal, and virtually no HT product was expressed. Thus the cytoplasmic tail of HT dets. the temporal order of action, and therefore dominance, of these two enzymes.

L5 ANSWER 10 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:599693 HCAPLUS

DOCUMENT NUMBER: 125:271382

TITLE: Modification of the cytoplasmic domain affects the subcellular localization of Golgi glycosyltransferases

AUTHOR(S): Yang, Wei; Pepperkok, Rainer; Bender, Patrick; Kreis, Thomas E.; Storrie, Brian

CORPORATE SOURCE: Virginia Polytechnic Institute, State University, Blacksburg, VA, 24061-0308, USA

SOURCE: Eur. J. Cell Biol. (**1996**), 71(1), 53-61
CODEN: EJCBDN; ISSN: 0171-9335

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Our goal was to engineer a Golgi **glycosyltransferase** epitope-tagged on its cytoplasmically exposed, short, N-terminal domain that gave normal subcellular localization. Partial replacement of the cytoplasmic tail of human .alpha.-2,6-sialyltransferase (SialylT) with the neg. charged myc or FLAG epitope resulted in almost complete mislocalization of the chimera expressed in Vero cells. A granular cytoplasmic staining pattern was seen by immunofluorescence. Spacing the neg. charged residues progressively outward from the neg. N-terminus resulted in increasingly more normal localization of myc or FLAG-tagged protein to a juxtanuclear Golgi-like distribution. Substitution of a neutrally charged VSV-G sequence for these tags resulted in normal localization of the chimera to the juxtanuclear Golgi region. Insertion of the myc epitope within the N-terminal domain of the short form of bovine .beta.-1,4-galactosyltransferase (GalT) gave a **chimeric protein** that mislocalized in BHK cells. No signal was detected with a monoclonal anti-epitope antibody indicating that the myc epitope was masked. Placement of myc or FLAG epitopes at the NH2-terminus of human N-acetylglucosaminyltransferase I (GlcNAc-T) resulted in **chimeric proteins** that in Vero cells displayed little

Golgi localization. We conclude that positioning of neg. charge, in particular, close to the membrane, typically produces a failure of type II Golgi **glycosyltransferases** to exit the endoplasmic reticulum/cis-Golgi network, presumably due to quality control mechanisms. These **proteins** may be successfully epitope-tagged on their N-terminal domain either using a neutral or pos. charged sequence or spacing any neg. charged sequence out from the membrane.

L5 ANSWER 11 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:351599 HCAPLUS
DOCUMENT NUMBER: 125:51989
TITLE: Immunodetection of .alpha.1-3 fucosyltransferase (FucT-V)
AUTHOR(S): Borsig, Lubor; Kleene, Ralf; Dinter, Andre; Berger, Eric G.
CORPORATE SOURCE: Institute Physiology, University Zurich, Zurich, Switz.
SOURCE: Eur. J. Cell Biol. (**1996**), 70(1), 42-53
CODEN: EJCBDN; ISSN: 0171-9335
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The fucosyltransferases constitute a family of **glycosyltransferases** incorporating fucose residues into glycoprotein or glycolipid glycans. They afford one of the possible termination steps of glycoconjugate biosynthesis creating the sialyl Lewisx or sialyl Lewisn determinant, which play an important role in cell-cell interaction. Whereas cDNA, chromosomal localization, and kinetic properties of a no. of fucosyltransferases are known, immunocytochem. localization and trafficking studies have been delayed because of the lack of specific antibodies due to the pronounced homol. of .alpha.1,3-fucosyltransferases III, V, and VI. Here, the development and characterization of monospecific polyclonal antibodies to .alpha.1-3-fucosyltransferase V (FucT-V) and their application for immunodetection in transfected cells are reported. Antisera against FucT-V were raised in 2 different ways: 1st by producing a **fusion protein** .beta.-galactosidase-FucT-V in Escherichia coli, and by synthesizing a peptide stretch specific for FucT-V. Polyclonal antisera were raised against each of both antigens and characterized by ELISA, neutralization of activity, immunoblotting, immunofluorescence, and immunopptn. of metabolically labeled COS cells, transiently transfected with cDNA encoding FucT-V. Both antibodies recognized only FucT-V. No crossreactivity to FucT-III or FucT-VI was obsd. FucT-V was localized mainly to the Golgi app. by colocalization with .beta.1,4-galactosyltransferase, and to the cell surface of COS, CHO, and HeLa cells. Expression of FucT-V in COS cells revealed 3 isoforms of 58, 53 and 50 kDa, resp. These size differences arose by posttranslational modifications, as shown by pulse-chase expts. The results indicate that .alpha.1-3-fucosyltransferase is a Golgi-assocd. enzyme and suggest its possible occurrence on the cell surface.

L5 ANSWER 12 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:180589 HCAPLUS
DOCUMENT NUMBER: 124:226128
TITLE: Localization of a yeast early Golgi mannosyltransferase, Och1p, involves retrograde transport
AUTHOR(S): Harris, Sandra L.; Waters, M. Gerard
CORPORATE SOURCE: Dep. Molecular Biology, Princeton Univ., Princeton, NJ, 08544, USA
SOURCE: J. Cell Biol. (**1996**), 132(6), 985-98
CODEN: JCLBA3; ISSN: 0021-9525
DOCUMENT TYPE: Journal
LANGUAGE: English
AB To analyze the mechanism of integral membrane protein localization in the

early Golgi app. of Saccharomyces cerevisiae, the authors have used Och1p, a cis-Golgi mannosyltransferase. A series of influenza virus hemagglutinin (HA) epitope-tagged **fusion proteins** was constructed in which invertase is appended to the Golgi-luminal carboxy terminus of full-length Och1p. Several constructs included a Kex2p cleavage site between the Och1p and invertase moieties to monitor transit to the Kex2p-contg. TGN. Cells expressing an Och1p-invertase fusion do not secrete invertase, but those expressing an Och1p-Kex2p site-invertase **fusion protein** secrete high levels of invertase in a Kex2p-dependent manner. The Och1p-Kex2p site-invertase **fusion protein** is cleaved with a half-time of 5 min, and the process proceeds to completion. Before cleavage the protein receives glycosyl modifications indicative of passage through the medial- and trans-Golgi; therefore, cleavage occurs after ordered anterograde transport through the Golgi to the TGN. Transit to distal compartments is not induced by the invertase moiety, since noninvertase fusion constructs encounter the same **glycosyltransferases** and Kex2p as well. The Och1p-HA moiety, irresp. of whether it is generated by cleavage of the **fusion protein** in the TGN or synthesized de novo, is degraded with a half-time of about 60 min. Thus, the half-time of degrdn. is 12-fold longer than the time required to reach the TGN. At steady state, de novo-synthesized and TGN-generated HA epitope-tagged Och1p reside in a compartment with a buoyant d. identical to that of wild-type Och1p and distinct from that of the vacuole or the TGN. Finally, och1 null cells that express an Och1p fusion construct known to rapidly encounter the TGN glycosylate invertase to the same extent as wild-type cells, indicating that they have phenotypically wild-type Och1p activity. These results led the authors to propose a model for Och1p-HA localization that involves movement to distal compartments, at least as far as the TGN, followed by retrieval to the cis compartment, presumably by vesicular transport.

L5 ANSWER 13 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:117724 HCAPLUS
DOCUMENT NUMBER: 124:166738
TITLE: Golgi localization and in vivo activity of a mammalian glycosyltransferase (human .beta.1,4-galactosyltransferase) in yeast
AUTHOR(S): Schwientek, Tilo; Narimatsu, Hisashi; Ernst, Joachim F.
CORPORATE SOURCE: Inst. Mikrobiol., Heinrich-Heine-Univ. Duesseldorf, Duesseldorf, D-40225, Germany
SOURCE: J. Biol. Chem. (**1996**), 271(7), 3398-405
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Gene fusions encoding the membrane anchor region of yeast .alpha.1,2-mannosyltransferase (Mnt1p) fused to human .beta.1,4-galactosyltransferase (Gal-Tf) were constructed and expressed in the yeast Saccharomyces cerevisiae. **Fusion proteins** contg. 82 or only 36 N-terminal residues of Mnt1p were produced and quant. N-glycosylated; glycosyl chains were shown to contain .alpha.1,6-, but not .alpha.1,3-mannose determinants, a structure typical for an early Golgi compartment. A final Golgi localization of both fusions was confirmed by sucrose gradient fractionations, in which Gal-Tf activity cofractionated with Golgi Mnt1p activity, as well as by immunocytol. localization expts. using a monoclonal anti-Gal-Tf antibody. In an in vitro Gal-Tf enzymic assay the Mnt1/Gal-Tf fusion and sol. human Gal-Tf had comparable Km values for UDP-Gal (about 45 .mu.M). To demonstrate in vivo activity of the Mnt1/Gal-Tf fusion the encoding plasmids were transformed in an alg1 mutant, which at the non-permissive temp. transfers short (GlcNAc)2 glycosyl chains to proteins. Using specific lectins the addn. of galactose to several yeast proteins in transformants could be detected. These results demonstrate that Gal-Tf, a mammalian **glycosyltransferase**, is functional in the mol. environment of the

yeast Golgi, indicating conservation between yeast and human cells. The in vivo function of human Gal-Tf indicates that the yeast Golgi is accessible for UDP-Gal and suggests strategies for the construction of yeast strains, in which desired glycoforms of heterologous proteins are produced.

L5 ANSWER 14 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:81591 HCAPLUS
DOCUMENT NUMBER: 124:127138
TITLE: Glucan-binding proteins, especially **glycosyltransferase**, and use of **fusion proteins** to prevent dental plaque and teeth staining
INVENTOR(S): Kuramitsu, Howard Kikuo; Schilling, Kurt Matthew
PATENT ASSIGNEE(S): Unilever NV, Neth.; Unilever PLC
SOURCE: PCT Int. Appl., 44 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
WO 9531556	A1	19951123	WO 1995-GB1070	19950511	<--
W: JP					
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE					
EP 759081	A1	19970226	EP 1995-918091	19950511	<--
R: DE, FR, GB, IT					
JP 10500127	T2	19980106	JP 1995-529437	19950511	

PRIORITY APPLN. INFO.: GB 1994-9387 19940511
WO 1995-GB1070 19950511

AB Polypeptides with specific binding affinity for glucan - esp. the glucan-binding domain of glycosyl transferase enzyme - is utilized in a compn. for oral care. The polypeptide may block the binding sites in dental plaque where glycosyl transferase would bind and generate more plaque, or it may be conjugated to - and provide targeted delivery of - an antiplaque or antistain agent.

L5 ANSWER 15 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:589699 HCAPLUS
DOCUMENT NUMBER: 123:193332
TITLE: A second ABC transporter is involved in oleandomycin resistance and its secretion by Streptomyces antibioticus
AUTHOR(S): Olano, Carlos; Rodriguez, Ana Maria; Mendez, Carmen; Salas, Jose A.
CORPORATE SOURCE: Inst. Univ. Biotecnologia, Univ. Oviedo, Oviedo, 33006, Spain
SOURCE: Mol. Microbiol. (**1995**), 16(2), 333-43
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A 3.2-kb SstI-SphI DNA fragment of S. antibioticus, an oleandomycin producer, conferring resistance to oleandomycin was sequenced and found to contain an open reading frame of 1710 bp (oleB). Its deduced gene product (OleB) showed a high degree of similarity with other proteins belonging to the ABC-transporter superfamily, including the gene product of another oleandomycin-resistance gene (OleC). The OleB protein contains 2 ATP-binding domains, each of .apprx.200 amino acids in length, and no hydrophobic transmembrane regions. Functional anal. of the oleB gene was carried out by deleting specific regions of the gene and assaying for oleandomycin resistance. These expts. showed that either the 1st or the 2nd half of the gene contg. only 1 ATP-binding domain was sufficient to

confer resistance to oleandomycin. The gene oleB was expressed in Escherichia coli fused to a maltose-binding protein (MBP) using the pMal-c2 vector. The MBP-OleB hybrid protein was purified by affinity chromatog. on an amylose resin and polyclonal antibodies were raised against the **fusion protein**. These were used to monitor the biosynthesis and phys. location of OleB during growth. By Western anal., the OleB protein was detected both in the sol. and in the membrane fraction and its synthesis paralleled oleandomycin biosynthesis. A Streptomyces albus strain, contg. both a **glycosyltransferase** (OleD) able to inactivate oleandomycin and the OleB protein, was capable of glycosylating oleandomycin and secreting the inactive glycosylated mol. It is proposed that OleB constitutes the secretion system by which oleandomycin or its inactive glycosylated form could be secreted by S. antibioticus.

L5 ANSWER 16 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:574279 HCAPLUS
DOCUMENT NUMBER: 123:140108
TITLE: Cell type differences in Golgi retention signals for transmembrane proteins
AUTHOR(S): Tang, Bor Luen; Low, Seng Hui; Wong, Siew Heng; Hong, Wanjin
CORPORATE SOURCE: Institute of Molecular and Cell Biology, National University of Singapore, Singapore, 0511, Singapore
SOURCE: Eur. J. Cell Biol. (**1995**), 66(4), 365-74
CODEN: EJCBDN; ISSN: 0171-9335
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The transmembrane domain of Golgi resident **proteins** such as .beta.-galactoside .alpha.2,6-sialyltransferase (ST) and N-acetylglucosaminyltransferase 1 (NT) contain a Golgi retention signal which confers Golgi retention to reporter **proteins** appended to them in the appropriate context. Thus, chimeras of the cell surface protein dipeptidyl peptidase IV contg. the transmembrane domain of ST and NT are retained in the Golgi app. in MDCK and COS cells, as assessed by indirect immunofluorescence microscopy. Transfection of these **chimeric** constructs into CHO cells, however, results in their transport to vesicular structures which do not colocalize with that of an endogenous Golgi marker, mannosidase II. Furthermore, the staining pattern of these structures are not affected by brefeldin A. Biochem. anal. of the transgene products in pulse-chase expts. revealed that the **chimeric proteins** eventually become resistant to endoglycosidase H, suggesting that they are transported beyond the medial Golgi and therefore the vesicular structures are likely to be post-Golgi. The vesicular structures colocalized well with a lysosomal marker, cathepsin D, and also with internalized FITC-dextran chased into the lysosomal compartment. Monitoring the cell surface appearance of the **chimeric protein** suggests that the majority is transported directly to the lysosomal compartment. Golgi retention can be completely restored for ST and improved for NT by the inclusion of sequences flanking the transmembrane domain. Our results reflect cell type differences in the interpretation of the transmembrane domain Golgi retention signal, established that general Golgi retention of type II **glycosyltransferases** requires the hydrophilic flanking sequence as well as the transmembrane domain, and demonstrate that **proteins** which escape Golgi retention may be channeled to the lysosomal pathway.

L5 ANSWER 17 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:440702 HCAPLUS
DOCUMENT NUMBER: 122:182984
TITLE: Golgi localization in yeast is mediated by the membrane anchor region of rat liver sialyltransferase
AUTHOR(S): Schwientek, Tilo; Lorenz, Claudia; Ernst, Joachim F.
CORPORATE SOURCE: Inst. Mikrobiologie, Heinrich-Heine-Univ. Duesseldorf,

Duesseldorf, D-40225, Germany
SOURCE: J. Biol. Chem. (**1995**), 270(10), 5483-9
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB To investigate the function of the membrane anchor region of a mammalian **glycosyltransferase** in yeast we constructed a fusion gene that encodes the 34 amino-terminal residues of rat liver .beta.-galactoside .alpha.-2,6-sialyl-transferase (EC 2.4.99.1) (ST) fused to the mature form of yeast invertase. Transformants of Saccharomyces cerevisiae expressing the fusion gene produced an intracellular heterogeneously N-glycosylated **fusion protein** of intermediate mol. wt. between the core and fully extended N-glycosylated form of invertase, suggesting a post-endoplasmic reticulum (ER) localization. In two types of cell fractionation using sucrose d. gradients the ST-invertase **fusion protein** cofractionated with Golgi marker proteins, whereas a minor fraction (about 30%) comigrated with a vacuolar marker; ST-invertase was not detected in other cell fractions, including the ER and the plasma membrane. Consistent with Golgi localization, about 70% of the total amt. of the ST-invertase fusion was immunopptd. with an antibody directed against .alpha.-1,6-mannose linkages. The results demonstrate that the membrane anchor region of a mammalian type II **glycosyltransferase** is able to target a protein to the secretory pathway and to a Golgi compartment of the yeast S. cerevisiae, indicating conservation of targeting mechanisms between higher and lower eukaryotes. Since typical yeast Golgi localization signals are missing in the ST-membrane anchor region the results also suggest that yeast as mammalian cells utilize diverse mechanisms to direct proteins to the Golgi.

L5 ANSWER 18 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:128404 HCAPLUS
DOCUMENT NUMBER: 120:128404
TITLE: Expression of deletion constructs of bovine .beta.-1,4-galactosyltransferase in Escherichia coli: Importance of Cys134 for its activity
AUTHOR(S): Boeggeman, Elizabeth E.; Balaji, Petety V.; Sethi, Navin; Masibay, Arni S.; Qasba, Pradman K.
CORPORATE SOURCE: Lab. Math. Biol., Natl. Cancer Inst., Bethesda, MD, 20892, USA
SOURCE: Protein Eng. (**1993**), 6(7), 779-85
CODEN: PRENE9; ISSN: 0269-2139
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Bovine acetylglucosamine .beta.-1,4-galactosyltransferase (EC 2.4.1.90) (I) belongs to the **glycosyltransferase** family and as such shares a general topol.: an N-terminal cytoplasmic tail, a signal anchor followed by a stem region, and a catalytic domain at the C-terminal end of the protein. CDNA constructs of the N-terminal-deleted forms of I were prepd. in a pGEX-2T vector and expressed in E. coli as glutathione S-transferase (GST) **fusion proteins**. Recombinant proteins accumulated within inclusion bodies as insol. aggregates that were solubilized in 5M guanidine-HCl and required an oxido-shuffling reagent for regeneration of the enzyme activity. Recombinant I, devoid of the GST domain, had 30-85% of the specific activity of bovine milk I with apparent Km values for N-acetylglucosamine and UDP-galactose similar to those of milk I. Deletion analyses showed that both the I and lactose synthetase activities remained intact even in the absence of the 1st 129 residues (pGT-d129). The activities were lost when either deletions extended up to residue 142 (pGT-d142) or when Cys-134 was mutated to Ser (pGT-d129C134S). These results suggested that the formation of a disulfide bond involving Cys-134 holds the protein in a conformation that is required for enzymic activity.

L5 ANSWER 19 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1993:646599 HCAPLUS
DOCUMENT NUMBER: 119:246599
TITLE: Specific sequences in the signal anchor of the .beta.-galactoside .alpha.-2,6-sialyltransferase are not essential for Golgi localization. Membrane flanking sequences may specify Golgi retention
AUTHOR(S): Dahdal, Refka Y.; Colley, Karen J.
CORPORATE SOURCE: Coll. Med., Univ. Illinois, Chicago, IL, 60612, USA
SOURCE: J. Biol. Chem. (**1993**), 268(35), 26310-19
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The .beta.-galactoside .alpha.-2,6-sialyltransferase is a trans Golgi/trans Golgi network **glycosyltransferase** which adds sialic acid residues to Asn-linked oligosaccharides of glycoproteins. Previous results suggested that the sialyltransferase stem and signal anchor including flanking sequences may be 2 independent Golgi retention regions. However, other expts. demonstrated that the sequence of the signal anchor itself was not important. To investigate whether the sialyltransferase signal anchor was necessary and sufficient for Golgi retention, several mutant and **chimeric proteins** were expressed and localized in Cos-1 and CHO cells. The signal anchor and flanking sequences were able to retain the sialyltransferase catalytic domain in the Golgi. However, efficient Golgi retention was still obsd. when the signal anchor was altered or entirely replaced in either the presence or absence of most of the luminal stem region. **Chimeric proteins** consisting of the sialyltransferase cytoplasmic tail and signal anchor fused to the extracellular domains of 2 different cell surface **proteins** demonstrated poor Golgi retention. A significant increase in the Golgi retention of 1 of these chimeras was obsd. when 2 lysines were placed next to the signal anchor on the luminal side. Taken together these results suggest that the sialyltransferase signal anchor is not necessary or sufficient for Golgi retention, rather, appropriately spaced cytoplasmic and luminal flanking sequences are the important elements of the sialyltransferase Golgi retention region.

L5 ANSWER 20 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:464389 HCAPLUS
DOCUMENT NUMBER: 119:64389
TITLE: Expression cloning of a cDNA encoding UDP-GlcNAc:Gal.beta.1-3-GalNAc-R (GlcNAc to GalNAc) .beta.1-6GlcNAc transferase by gene transfer into CHO cells expressing polyoma large tumor antigen
AUTHOR(S): Bierhuizen, Marti F. A.; Fukuda, Minoru
CORPORATE SOURCE: Cancer Res. Cent., La Jolla Cancer Res. Found., La Jolla, CA, 92037, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (**1992**), 89(19), 9326-30
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A cDNA encoding UDP-GlcNAc:Gal.beta.1-3GalNAc-R (GlcNA to GalNAc) .beta.1-6GlcNAc transferase (EC 2.4.1.102), which forms crit. branches in O-glycans, has been isolated by an expression cloning approach using Chinese hamster ovary (CHO) cells. Increased activity of this enzyme and the concomitant occurrence of the O-glycan core 2 structure [Gal.beta.1-3(GlcNAc.beta.1-6)GalNAc] has been obsd. in a variety of biol. processes, such as T-cell activation and immunodeficiency due to the Wiskott-Aldrich syndrome and AIDS. Since CHO cells do not express this enzyme, CHO cell lines were established to stably express polyoma large tumor (T) antigen, which enables transient expression cloning. Because the antibody used was found to detect most efficiently the oligosaccharide products attached to leukosialin, the CHO cells were also stably transfected with leukosialin cDNA. By using this particular CHO cell

line, a cDNA that encodes a protein detg. the formation of the core 2 structure was isolated from an HL-60 cDNA library. The cDNA sequence predicts a protein with type II membrane topol., as has been found for all other mammalian **glycosyltransferases** cloned to date. The expression of the presumed catalytic domain as a **fusion protein** with the IgG binding domain of protein A permitted unequivocal demonstration that the cDNA encodes the core 2 .beta.-1,6-N-acetylglucosaminyltransferase, the enzyme responsible for the formation of Gal.beta.1-3(GlcNAc.beta.1-6)GalNAc structures. No activity with this enzyme was detected toward the acceptors for the .beta.1-6GlcNAc transferases.